MATING BEHAVIOUR AND FEMALE SPERM STORAGE IN *PHOLCUS PHALANGIOIDES* (FUESSLIN) (ARANEAE)

G. UHL

Uhl, G. 1993 11 11: Mating behaviour and female sperm storage in *Pholcus phalangioides* (Fuesslin) (Araneae). *Memoirs of the Queensland Museum* 33(2): 667-674. Brisbane. ISSN 0079-8835.

Females of *Pholcus phalangioides* do not possess receptacula seminis but store transferred spermatozoa in their genital cavity. The spermatozoa are embedded in glandular secretion that is discharged from two accessory glands situated in the posterior wall of the genital cavity. The gland cells belong to a complex type of class 3 cells according to the classification of Noirot and Quennedy (1974, 1991). With the sperm mass of a single copulation the females are able to fertilize several batches of eggs, although the sperm might be easily washed out with the passage of the first batch of eggs (Forster, 1980). Nevertheless, females allow repeated copulations. The first copulation usually took over an hour but subsequent copulations lasted only a few minutes, no matter whether the female mated with the same or with a different male. Copulations after egg-laying tended to be long. Male spiders might achieve reproductive advantage in copulating with any female they meet and females might have an interest in filling up their storage capacities.

Pholcus phalangioides Weibchen besitzen keine Receptacula seminis im üblichen Sinne, sondern speichern die während einer Kopulation übertragenen Spermien im Hohlraum des Uterus externus. Die Spermien werden dort in ein Sekret eingelagert, welches von zwei akzessorischen Drüsen produziert wird. Diese Drüsen befinden sich in der posterioren Wand des Uterus externus. Die Drüsenzellen sind nach einem Klassifikationssystem, das für epidermale Drüsenzellen von Insekten erstellt wurde (Noirot and Quennedy, 1974, 1991), einem komplizierten Typ der Klasse 3 zuzuordnen. Mit den Spermien, die während einer einzigen Kopulation übertragen wurden, können die Weibchen mehrere Eigelege befruchten (Uhl, in press a), entgegen der Annahme, die Spermien könnten während der ersten Eiablage leicht ausgewaschen werden (Forster, 1980). Die Weibchen lassen dennoch mehrere Kopulationen zu, wobei die erste Kopulation gewöhnlich über eine Stunde dauert, jede weitere Kopulation schon nach wenigen Minuten abgebrochen wird, unabhängig davon ob es sich um das selbe oder um ein neues Männchen handelt. Nach einer Eiablage lassen die Weibchen wieder lange Kopulationen zu. Für die Männchen mag es von Vorteil sein sich mit jedem Weibchen zu paaren dem sie begegen, und Weibchen könnten ein Interesse daran haben ihre Speicherkapazität voll auszuschöpfen. □*Araneae, Pholcidae, Pholcus phalangioides, sperm storage, glands, secretion, ultrastructure, repeated matings, copulation duration.*

Gabriele Uhl, Institut für Biologie I (Zoologie), Universität Freiburg i.Br., Albertstraße 21a, 7800 Freiburg i.Br., Germany: 28 October, 1992.

Most female spiders store sperm within storage structures that are spatially separated from the genital cavity. Some 'primitive' spider families such as Diguetidae, Liphistiidae, Archaeidae and Pholcidae retain the sperm mass within the genital cavity itself (Forster, 1980). Forster assumed that the bursal storage mode has little survival value as the sperm mass gets flushed out with the passage of the eggs and therefore, storing the spermatozoa in spatially separated storage structures would eliminate the need for repeated insemination. Despite the bursal storage mode, female *Pholcus phalangioides* (Fuesslin) are able to fertizile numerous batches of eggs with the sperm of a single insemination (Uhl, in press a). However, the females probably do not rely on the amount and fertility of the spermatozoa transferred during a single copulation. This would be risky as the spermatozoa might be defective or insufficient. I expect the females to fill up their storage capacity by means of repeated matings at least after egg-laying when few clumps of spermatozoa remain in the genital cavity after oviposition. However, if the female does not have the opportunity to copulate repeatedly, the stored spermatozoa can be sufficient for fertilizing following egg batches successfully. This study will also give a brief morphological account on the bursal storage mode in *P. phalangioides* and will present histological and ultrastructural findings on the glandular tissue that exudes its product into

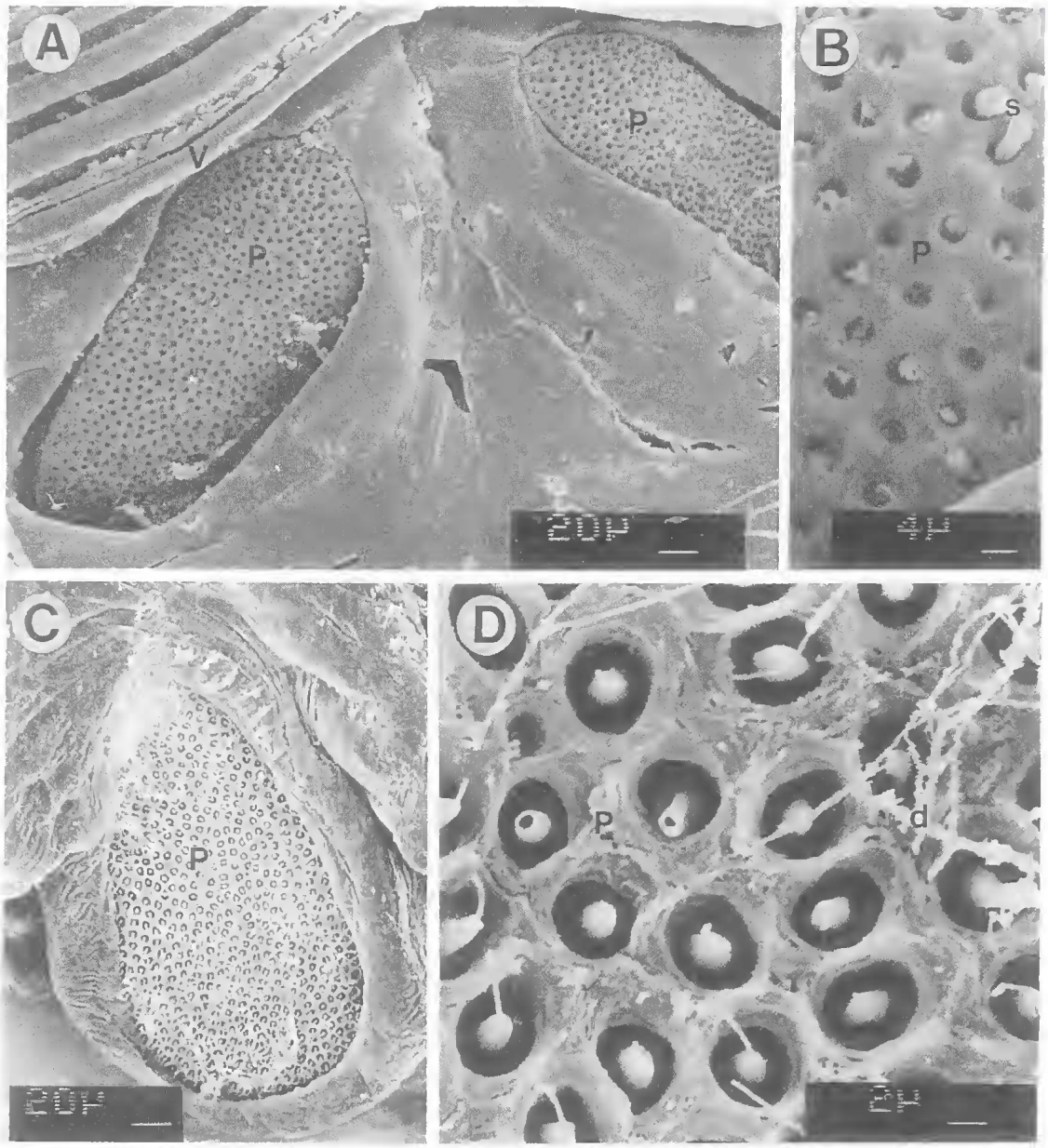


FIG. 1. Dorsal wall of genital cavity of *P. phalangioides*. A, Pore plates viewed from genital cavity; B, Pores that exude secretion, C, One pore plate from its dorsal side, glandular tissue removed; D, cuticular ductules of accessory glands.

the genital cavity for sperm storage. For more detailed information see Uhl (in press b, c).

MATERIAL AND METHODS

Juvenile *Pholcus phalangioides* were reared individually in the lab. To investigate mating behaviour the spiders were kept in couples in plastic boxes (16.5x9x6.5cm) and their behaviour was recorded day and night on video tape.

Five different experimental set-ups were used in order to answer the indicated questions:

1. Duration of copulation: Virgin females were offered unexperienced males.

2. Copulation duration with sperm-depleted males: Virgin females were offered recently experienced males (1/2 hour after termination of copulation).

3. Repeated matings with same partner: Previously virgin females were kept with previously unexperienced males for up to 20 days.

4. Repeated matings with changing partner: 1/2 hour after their first copulation females were offered unexperienced males.

In order to check the influence of box-size on mating behaviour 4 experiments were carried out using 2.5 times bigger boxes (18x12x11.5cm).

5. Duration of copulation after egg-laying: Post-oviposition females that had mated once were allowed to copulate again with unexperienced males.

For SEM studies adult females were anaesthetized, dissected and fixed in 70% ethanol or Bouin. In order to investigate the sclerotized parts of the female genital tract, the female genitalia were put in 5% NaOH solution until the soft parts were dissolved. Some genitalia were opened or cut with a sharp razorblade to locate the sperm mass in the genital cavity. They were dehydrated in ethanol, CP-dried, sputter coated with gold and examined in a Zeiss Semco Nanolab 7.

For light- and electron microscropy the spiders were anaesthetized and dissected in glutaraldehyde. After fixation in 2% osmium tetroxide/-glutaraldehyde, they were post-fixed in osmic acid (modified after Franke *et al.*, 1969), dehydrated in graded series of alcohol followed by propylene oxide and embedded in Epon. The semithin sections (0.7-1μm) were cut with glass knives on a Reichert OmU3 and stained with toluidin. Ultrathin sections were cut with glass knives and diamond knife. They were stained with uranyl acetate, counterstained with lead citrate and examined in a Zeiss EM9 electron microscope.

RESULTS

Mating Behaviour

1. Virgin females copulated with unexperienced males over an hour ($\bar{x}$=64.5 minutes; sd= 26.6; shortest duration: 16 min, longest duration: 122 min; n=42). This supports findings of Reagan and Reagan (1989) who investigated 104 pairs (mean copulation duration: 72.3 min; sd=43.3; shortest: 10 min, longest: 304 min).

2. Five males that had mated with virgin females (long copulation I) were brought half an hour after copulation to another virgin female. All

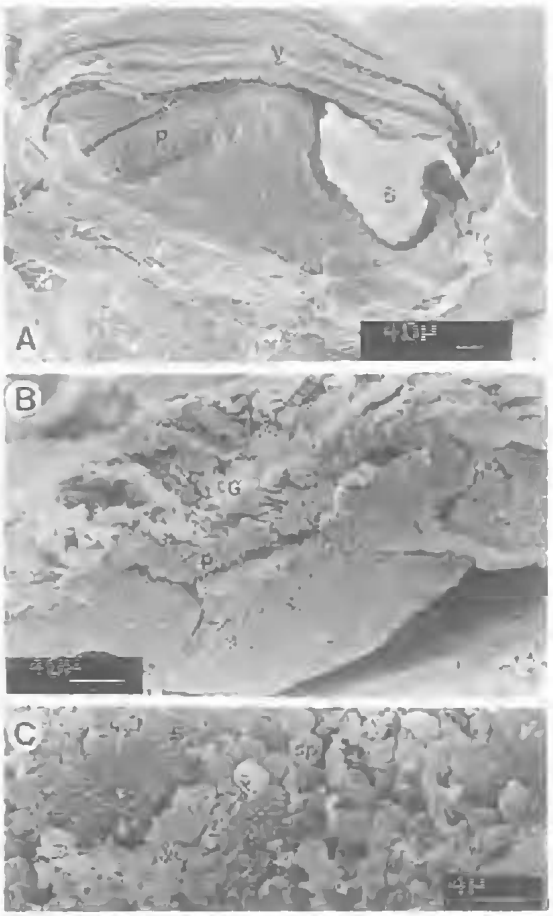


FIG. 2. A, Genital plate flipped back, dorsal wall of genital cavity revealed. Two pore plates, one concealed by secretory 'plug'; B, secretory 'plug' in genital cavity cut sagittally; C, Sperm mass in female secretion in female genital tract.

copulations were long ($\bar{x}$: 56.8 minutes). The males probably refill their copulatory organs prior to the second copulation. The filling was observed in only one case by chance and was not detectable on the video recording.

3. Unexperienced males and females were kept in pairs up to 20 days. Six females out of 12 allowed copulation from time to time: one female copulated twice, four females copulated 3 times, one female copulated five times. The second and the following copulations were always very short, they took only 2 to 5 minutes. Copulation duration tended to decrease in successive matings.

4. Unexperienced males were brought to females that had already mated once (long copulation I). In 9 of 14 cases the females allowed

Experimental set-up	n	Copulation duration (min.)
1. Virgin ♀ with unexperienced ♂	42	64.5 (16-122; 26.6)
2. Virgin ♀ with recently experienced ♂	5	56.8 (37-75; 13.9)
3. Second copulation with same ♂	6*	3.6 (2-5; 1.02)
4. Second copulation with unexperienced ♂	9*	2.6 (1.5-5; 1.0)
5. Post-oviposition ♀ with unexperienced ♂	7	59.6 (21-103; 31.4)

TABLE 1. Mating behaviour in *P. phalangioides* as a function of female reproductive history. * Only cases of copulation given. Range and standard deviation in parentheses.

further copulation. Again, second copulations lasted only a few minutes (1.5-5 min). Control experiments using bigger boxes showed that 3 females out of 4 allowed repeated matings (4.5; 2; 1 + 1 min).

5. Seven females that had mated once (long copulation I) and were kept separately afterwards, had access to males after oviposition. Copulation duration was long.

Copulation duration seems to depend on the reproductive history of the females (Table 1).

The Genital Cavity

The dorsal (posterior) wall of the genital cavity is characterized by two oval pore plates (Fig. 1A). The pore plates converge in direction of the ridges and grooves that make up the heavily sclerotized valve which separates the genital cavity from the oviducts. Both plates are perforated by pores of 3-5μm in diameter (Fig. 1B). The pores are in contact with gland cells that exhude their glandular secretions into the cavity (Fig. 1B).

The tissue-free pore plates reveal the canal zones of the glandular tissue when looked at from their dorsal side (Fig. 1C). Situated in cavities, cone-shaped hollow structures are apparent (Fig. 1D). These are the distal regions of the canals that open into the uterus externus as the pores of the pore plate. Proximally the canals change into thin ductules that exhibit a rough surface after 6-10μm (Fig. 1D).

Sperm Storage

The accessory glands discharge their products through the pores of the pore plates into the genital cavity and form two portions of secretory 'plugs'. (Fig. 2A). During copulation, the male transfers sperm mass into the female secretion (Fig. 2B). The spermatozoa are surrounded by individual secretory envelopes, they are coiled and inactive (Fig. 2C).

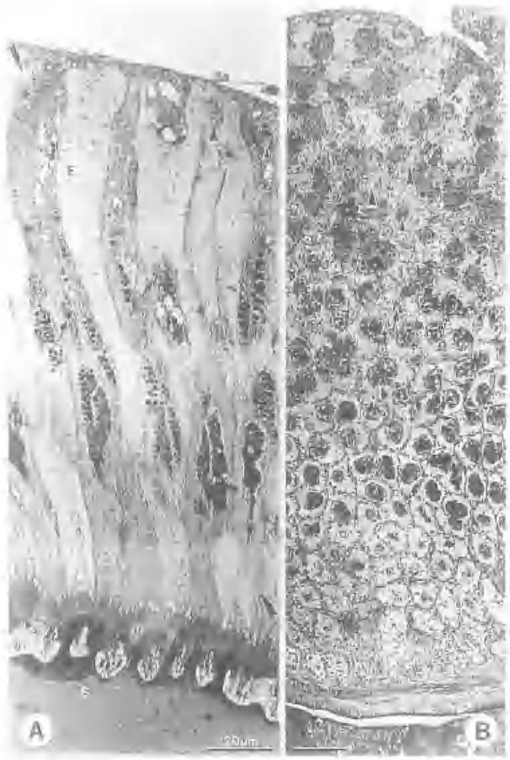


FIG. 3. Semithin section of accessory glands. A, Longitudinal section. Arrows mark nuclei of different cell types. Ba: basal lamina, E2: outer envelope cell, G: gland cell, m: microvilli, P: pore plate, s: secretion of the gland cells, S: secretion in genital cavity; B, Transverse section. Arrows and stars mark nuclei of different cell types. Scale lines: 20μm.

The Accessory Glands

The glandular tissue is composed of highly elongated cells (Fig. 3). Different cell types form the gland with various nuclei at different levels (Fig. 3A). The gland consists of lightly stained cells whose nuclei lie close to the basal lamina, and densely stained cells with more distal nuclei. Nuclei of other cell types lie mainly in the centre.

Secretory vesicles are apparent in the densely stained cells (the gland cells G). Accumulations of such vesicles are found in the apical second third of the glandular tissue. Each accumulation forms two portions of tightly packed secretory globules that discharge their contents into a common reservoir which is homogeneously coloured (Fig. 3A, B).

Bordering on the gland's orifices is a zone of

greyish coloration that is formed by the light coloured cells (outer envelope cells E2).

Histology and ultrastructure show that the glandular tissue includes many similar units, each provided with a cuticular ductule that leads to the pore plate (Fig. 3A). Each unit comprises two gland cells and two envelope cells. The two gland cells (G1, 2) join each other to form a common reservoir (Fig. 4, 5B). They are rich in granular endoplasmic reticulum (Fig. 5A), mitochondria and dense secretory vesicles and exhibit numerous golgi complexes in the supranuclear region. The vesicles vary in size (up to 1.5µm in diameter) and get more numerous in the distal cell region. They are enclosed in a close-fitting membrane which is obscured by the matching density of the mature granula. The inner envelope cell (E1) surrounds and partially separates the two gland cells (Fig. 5B, C, D) and forms the proximal part of the ductule (Fig. 4C). The outer envelope cell (E2) surrounds all of the previously mentioned cells. Its cytoplasm is poor in organelles (Fig. 5A, B, D). It produces the distal part of the ductule and forms numerous microvilli that gather round the ductule and the orifice (Fig.5E) and represent the greyish zone visible in the semithin section of Fig. 3A.

The gland cells and the outer envelope cell form a so-called basal labyrinth adjacent to the basal lamina (Figs 4, 5A). The glandular units are separated from each other by elongated epithelial cells.

DISCUSSION

The glandular units of the accessory glands in *P. phalangioides* belong to class 3 cells (Noirot and Quennedy, 1974, 1991). According to that classification, a gland cell is associated with a cuticular ductule that has been secreted by a 'canal' cell. In *P. phalangioides* however, there are two gland cells that are always connected by a common microvilli region, one inner and one outer envelope cell that form a double ensheathing of the gland cells. Moreover, both envelope cells take part in producing the canal that leads to the pore plate. Therefore, the glands studied here belong to a complicated type of class 3 cells.

There is some information on gland structures in female spider genitalia. The glands of the receptaculum seminis of Telemidae belong to class 1 type of gland cells (Lopez and Juberthie-Jupeau, 1983), and in some Theraphosidae De Carlo (1973) stated a class 1 composition. However, the light microscope study of Kovoor (1981) indi-

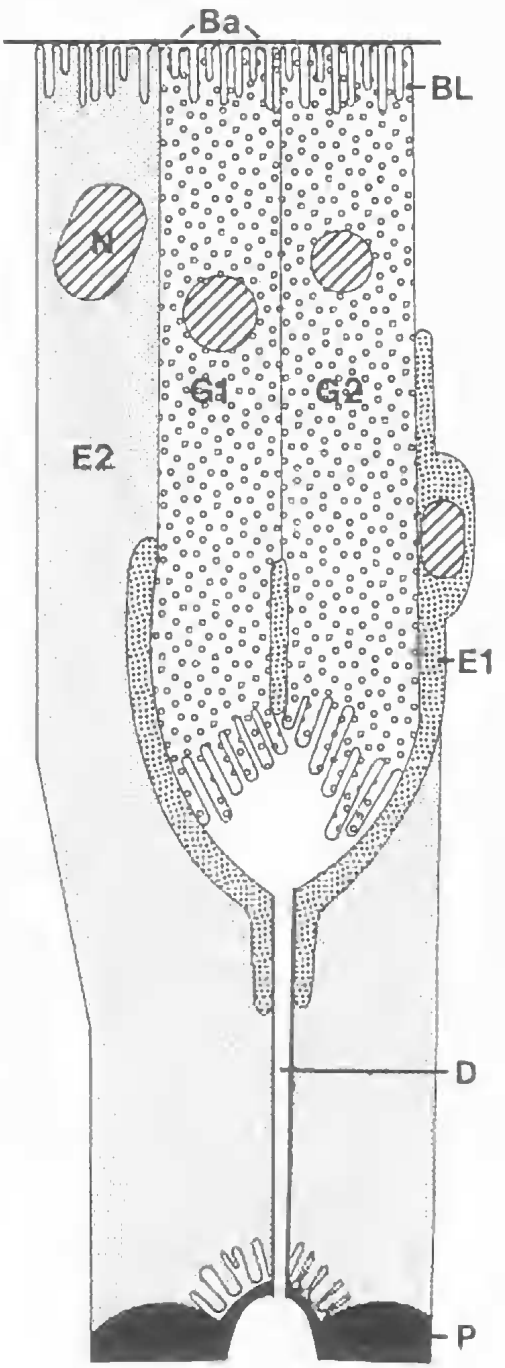
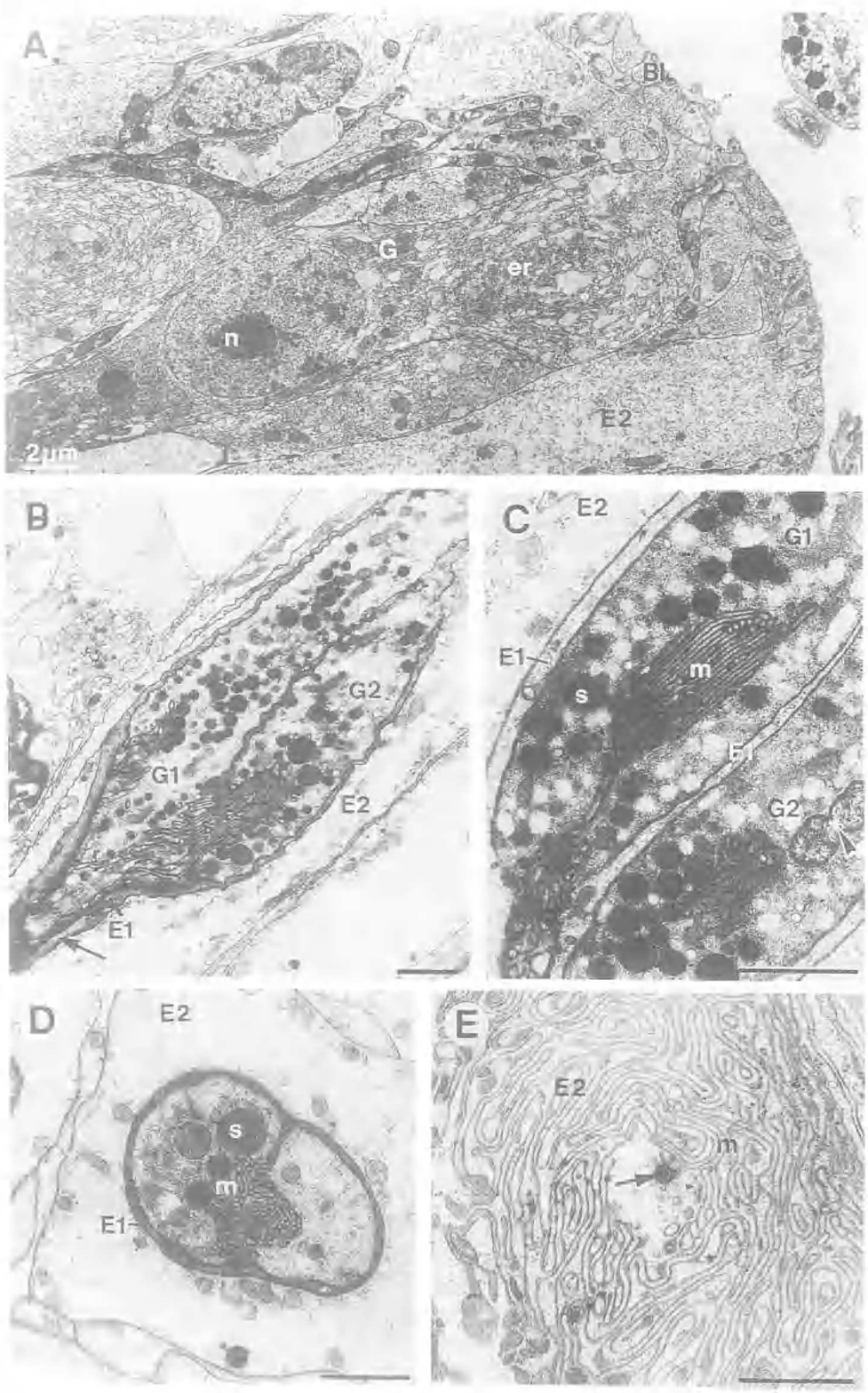


FIG. 4. Schematic reconstruction of one glandular unit of accessory glands. Ba: basal lamina, BL: basal labyrinth, D: ductule, E1: inner envelope cell, E2: outer envelope cell, G1, 2: gland cells, N: nucleus, P: pore plate.


A
Bl
G
er
n
E2
2 µm
B
G2
G1
E2
E1
C
E2
G1
E1
m
s
G2
D
E2
s
m
E1
E
E2
m

cates that a more complex gland composition as in *P. phalangioides* might not be exceptional.

The glandular units of *P. phalangioides* could function as a two-component-system, as the outer envelope cell shows conspicuous microvilli that surround the orifices of the ductules. This indicates secretory activity although the outer envelope cell contains no stainable secretory droplets or granules. Their product could have got lost during fixation or, the cells produce and release their product only on demand. Not all the secretory activities of cells are accompanied by microscopically detectable accumulation of the product in the cytoplasm. Apart from that, the outer envelope cell exhibits a basal labyrinth. It contributes to enhance the exchange of molecules between the haemolymph and the cells (Berridge and Oschmann, 1972) and characterizes active cells that take up or transfer material from or to the haemolymph.

The glandular secretions might serve various functions such as nutrition of the sperm (Coyle *et al.*, 1983; De Carlo, 1973; Engelhardt, 1910; Forster, 1980), pheromone production (Kovoor, 1981) or sperm displacement from the spermathecae into the genital cavity during oviposition (Forster *et al.*, 1987; Lopez, 1987; Lopez and Juberthie-Jupeau, 1983). Brignoli (1976) and Lopez and Juberthie-Jupeau (1983) considered activation of sperm prior to fertilization. The glandular tissue might be responsible for triggering activation via a secretory product that gets released exclusively before oviposition. Further, the females might achieve advantages from resorbing the sperm mass out of the genital cavity.

I consider the secretion in the female genital tract of *P. phalangioides* serves primarily as a depot for the sperm that guarantees successful storage as the onset of the female receptivity corresponds with the time needed to fill the genital cavity with glandular secretion (Uhl, in press a). Concerning any other possible functions, specific investigations are still lacking.

The bursal storage mode is considered a 'primitive' mode with little survival value as the spermatozoa are liable to be washed out during oviposition (Forster, 1980). Nevertheless, females of *P. phalangioides* succeed in producing several fertile batches of eggs after a single mating (Uhl, in press a).

Although females do not depend on repeated insemination, they allow further copulations. These always last only a few minutes in constrast to the first copulation that lasts over an hour and there is no apparent difference in copulation duration between second copulations with the same or with a different male. If new males are able to replace sperm of a previous male, longer copulations would be expected. Such short copulations may suggest that males are able to assess female virginity or reproductive history during insertion of their palpal structures and then decide on further investment of time and energy.

It has yet to be investigated whether successive copulations result in a transfer of spermatozoa at all, which will also give information on sperm precedence. Provided they transfer sperm and fertilise at least some eggs, it would be advantageous for males to mate with any female they meet. The females, on the other hand, can be expected to fill their storage structure with as many spermatozoa as possible to achieve the highest possible reproductive success. Depending on the amount of sperm already accumulated in the genital cavity females might allow further copulations and hence, decide on copulation duration. Indeed, there is some evidence that the female terminates copulation.

ACKNOWLEDGEMENTS

I wish to thank my supervisor Prof. Dr. P. Weygoldt (Freiburg) for making this work possible. Dr. M. Schmitt (Bonn) and two anonymous referees gave helpful comments on the manuscript. Without a grant from the Studienstiftung des Deutschen Volkes and additional support from the Organizing Committee participation at the XIIth Congress of Arachnology, Brisbane would have been impossible.

FIG. 5. Ultrathin sections of accessory glands. A) Longitudinal section. Nuclear region of gland cells. B, Longitudinal section. Two gland cells join to form common microvilli region, surrounded by two envelope cells forming first part of canal (arrow). C) Longitudinal section. Microvilli region. Arrow shows cleaving bacterium. D) Transversal section. Beginning of ductule. Gland cells joined, enveloped twice. E) Microvilli region of outer envelope cell close to pore plate. Small ductule (arrow); BL: basal labyrinth, E1: Inner envelope cell, E2: outer envelope cell, er: endoplasmatic reticulum, G1: gland cell 1, G2: gland cell 2, n: nucleus, m: microvilli, s: secretory droplets. All scale lines, 2μm.

LITERATURE CITED

BERRIDGE, M.J. & OSCHMAN, J.L. 1972. 'Transporting epithelia'. (Academic Press, New York, London).

BRIGNOLI, P.M. 1976. Ragni d'Italia XXIV. Note sulla morfologia dei genitali interni dei Segestriidae e cenni sulle specie italiane (Araneae). Fragmenta Entomologica 12: 19-62.

COYLE, F.A., HARRISON, F.W., MCGIMSEY, W.C. & PALMER, J.M. 1983. Observation on the structure and function of spermathecae in haplogyne spiders. Transactions of the American Microscopical Society 102: 272-280.

DE CARLO, J.M. 1973. Anatomia microscopica de las espermatecas de los generos *Grammostola* y *Acanthoscurria*. Physis Seccion C, Buenos Aires 32(85): 329-342.

ENGELHARDT, V. 1910. Beiträge zur Kenntnis der weiblichen Copulationsorgane einiger Spinnen. Zeitschrift für wissenschaftliche Zoologie 96: 32-117.

FORSTER, R.R. 1980. Evolution of the tarsal organ, the respiratory system and the female genitalia in spiders. Verhandlungen des 8.Internationalen Araehnologen Kongreß, Wien 1980: 269-283.

FORSTER, R.R., PLATNICK, N.I. & GRAY, M.R. 1987. A review of the spider superfamilies Hypochilidae and Austrochilidae (Arancae, Araneomorphae). Bulletin of the American Museum of Natural History 185: 1-116.

FRANKE, W.W., KRIEN, S. & BROWN, R.M. 1969. Simultaneous glutaraldehyde-osmium tetroxide fixation with postosmication, an improved fixation procedure for electron micoscropy of plant and animal cells. Histochemistry 19: 162-164.

KOVOOR, J. 1981. Une source probable de pheromone sexuelles: les glandes tégumentaires de la région génitale des femelles d'araignées. Atti della Società Toscana di Scienze Naturali, Memoire Serie B, Supplemento 88: 1-15.

LOPEZ, A. 1987. Glandular aspects of sexual biology. Pp. 121-131. In Nentwig, W. (ed.). 'Ecophysiology of spiders'. (Springer: Berlin, Heidelberg, New York).

LOPEZ, A. & JUBERTHIE-JUPEAU, L. 1983. Structure et ultrastructure de la spermathèque chez *Telema tenella* Simon (Araneae, Telemidae). Mémoires Biospéologiques 10: 413-419.

NOIROT, C. & QUENNEDY, A. 1974. Fine structure of insect epidermal glands. Annual Review of Entomology 19: 61-80.

1991. Glands, gland cells, glandular units: some comments on terminology and classification. Annales de la Societé Entomologique de France (N.S.) 27: 123-128.

REAGAN, N.L. & REAGAN, A.P. 1989. Mating and egg production in *Pholcus phalangioides*. American Arachnological Society, Newsletter 40.

UHL, G. (in press a). Sperm storage and repeated egg production in female *Pholcus phalangioides* (Fuesslin) (Araneae). Bulletin de la Societé Neuchâteloise des Sciences Naturelles. Actes de la XIIIème Colloque Européen d'Arachnologie, Neuchâtel 2.-6. Sept. 1991.

(in press b). Genital morphology and sperm storage in *Pholcus phalangioides* (Fuesslin) (Araneae). Acta Zoologica, Stockholm 74.

(in press c). Ultrastructure of the accessory glands in female genitalia of *Pholcus phalangioides* (Fuesslin) (Arancae Arachnida). Acta Zoologica, Stockholm 74.